



LC/MS QUANTITATION OF 25-HYDROXYVITAMIN D₂/D₃ IN HUMAN SERUM

APPLICATION NOTE

Mass Spec: expression CMS
LC Method: HPLC

In this application note, a simple and cost-effective SIM LC/MS analytical approach employing the Advion expression Compact Mass Spectrometer (CMS) is described for the quantitation of 25-hydroxyvitamin D₂/D₃ in human serum.

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INTRODUCTION

It is well known that vitamin D is important to bone health while its major physiological function is to maintain blood calcium and phosphorus levels within normal range, as well as to maintain metabolic functions that are essential for most life processes. Vitamin D deficiency is associated with osteoporosis and rickets, cardiovascular disease, diabetes, and autoimmune disorders. Its deficiency is also associated with depression, stroke, chronic pain, osteoarthritis, and many forms of cancer^[1-3].

The hydroxy metabolites of vitamin D are accepted indicators of vitamin D levels in human serum. The quantitative determination of these metabolites (25-hydroxyvitamin D₂ and D₃) is widely accepted as a means of assessing vitamin D deficiency status.

In this application note, a simple and cost-effective SIM LC/MS analytical approach employing the Advion **expression** Compact Mass Spectrometer (CMS) is described for the quantitation of 25-hydroxyvitamin D₂/D₃ in human serum.

METHODS

Sample Preparation

The solvents used in this application were purchased from Sigma-Aldrich (St. Louis, MO). 25-hydroxyvitamin D₂ and D₃, and D₆-25-hydroxyvitamin D₃ were purchased from Cerilliant (Round Rock, TX). The control serum with known amounts of 25-hydroxyvitamin D₂ and D₃ was purchased from UTAK Laboratories (Valencia, CA).

Extraction Procedure

500 µL of serum was mixed with 10 µL of internal standard solution (D₆-25-hydroxyvitamin D₃) followed with the addition of 40 µL saturated aqueous ZnSO₄ and 1 mL acetonitrile.

Liquid-liquid extraction was done with 2.5 mL hexane followed by centrifugation, removal, and concentration to dryness of the upper layer. The residue was reconstituted in 150 µL of MeOH/water (50/50; v/v).

MS Conditions

Ion Source: Positive APCI
Capillary Temp: 250 °C
Capillary Voltage: 150 V
Source Voltage Offset: 30 V
Source Voltage Span: 0 V
Gas Temperature: 350 °C
Corona Discharge: 5 µA

LC Conditions

Column: Agilent C18, 50 x 2.11 mm, 1.8 µ particles
Solvent A: 0.1% formic acid in water
Solvent B: 0.1% formic acid in acetonitrile
Flow Rate: 400 µL/min
Injection Volume: 25 µL

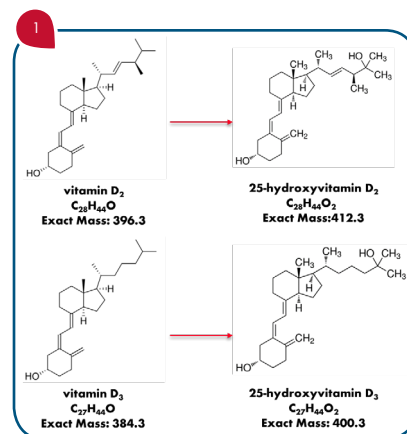


Figure 1: Chemical structures of Vitamin D₂ and D₃, and 25-hydroxyvitamin D₂ and D₃.

Gradient

Time (min)	B (%)
0.0	50
4.0	100
7.5	100
7.6	50
10	50

RESULTS

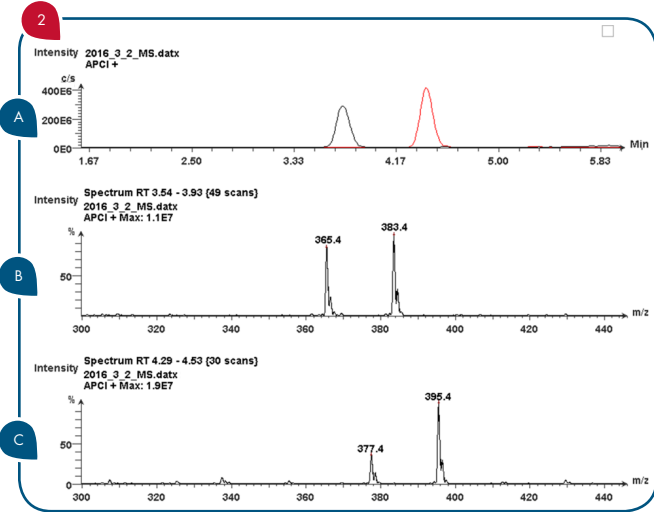


Figure 2: LC/MS Determination of 25-OH-Vitamin D₂/D₃. (A) Full-scan LC/MS total ion chromatogram (TIC) of standard 25-OH-Vitamin D₂ and D₃. (B) Mass spectrum of 25-OH-Vitamin D₃. (C) Mass spectrum of 25-OH-Vitamin D₂.

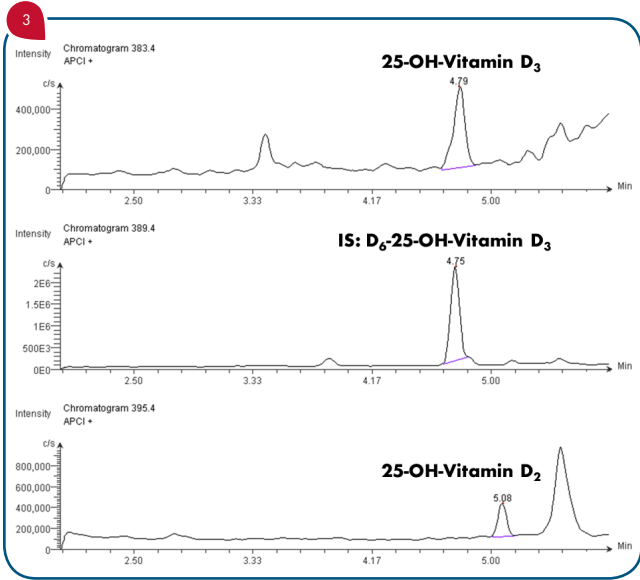


Figure 3: LC/SIM analysis of low-level control serum sample, which contains 10 ng/mL of 25-hydroxyvitamin D₂/D₃. The signal-to-noise ratio (S/N) = 63 for 25-hydroxyvitamin D₃ and S/N = 50 for 25-hydroxyvitamin D₂.

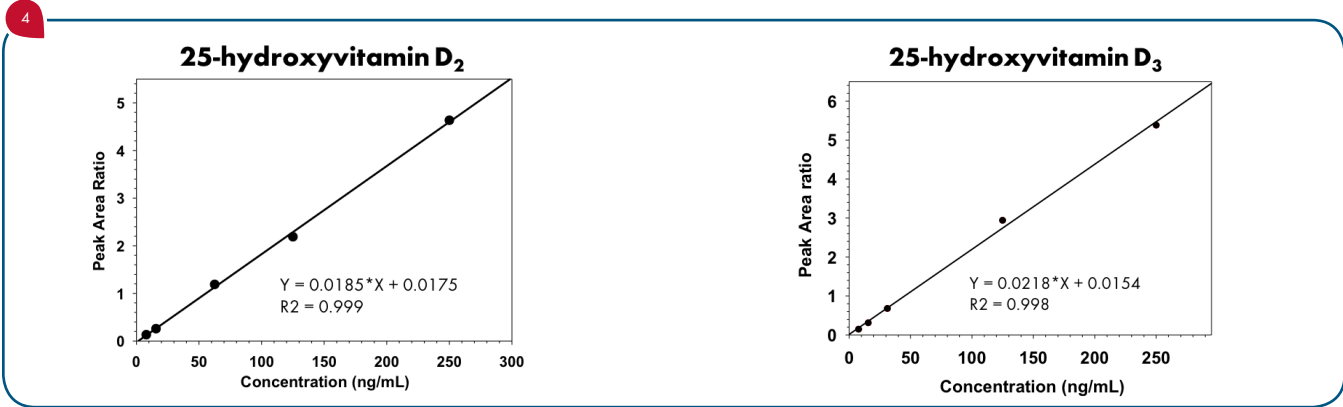


Figure 4: Calibration curve of 25-hydroxyvitamin D₂/D₃. D6-25-hydroxyvitamin D3 was used as an internal standard for quantitation. The linear concentration range with good linearity was from 7.8 to 250 ng/mL. This covers the normal reference range level in human serum. The calibration curves show an R² greater than 0.99. With an n=4 injections for each standard, the %CV was less than 10%. Accuracy averaged 90=110% for each standard shown.

Table 1: The commercially available control serum (UTAK VITAMIN D PLUS LOW) is reported to contain 25-hydroxyvitamin D₂ (12 ng/mL) and 25-hydroxyvitamin D₃ (10 ng/mL). Five separate analyses were performed in replicates of three. The measured values using the CMS with SIM LC/MS are consistent with the reported values from Cerilliant.

UTAK Vitamin D Plus Low (ng/mL)				
Analyte	Target Value	Verified Value	Expected Range	Measured Values with LC/CMS (5 replicates)
25-OH-Vitamin D ₂	10	12	10-14	11.9 ± 1.0
25-OH-Vitamin D ₃	10	10	9-12	10.2 ± 1.2

SUMMARY

The Advion expression CMS can be an effective tool for the quantitation determination of 25-hydroxyvitamin D₂/D₃ in human serum.

The linear dynamic range was from 8-250 ng/mL, which covers the normal reference range of vitamin D in human serum. The estimated quantitation level can be readily achieved down to at least 10 ng/mL.

The ease-of-use, affordability, and capability of the Advion expression CMS make it a viable option for serum 25-hydroxyvitamin-D analysis.

REFERENCES

^[1]Moyad, M.A., Holick, F. Vitamin D Deficiency. N Engl J Med 2007, 357:266-281

^[2]Moyad, M.A. Vitamin D: A rapid review. Urol Nurs. 2008 Oct; 28(5):343-9, 384

^[3]Svoren, B.M., Volkering, L.K., Wood, J.R., Laffel, L.M. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. J Pediatr. 2009 Jan;154(1):132-4